Factors Affecting Germination of *Puccinia jaceae* var. *solstitialis* Teliospores from Yellow Starthistle

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ABSTRACT

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The rust fungus *Puccinia jaceae* var. *solstitialis* is a candidate for biological control of yellow starthistle (YST). Part of the risk assessment includes determining if safflower seedlings are susceptible to infection by teliospores of *P. jaceae*. A protocol for germination of *P. jaceae* teliospores is needed to verify that teliospores used in comparative studies are viable. The protocol developed from this research has two steps: first, priming teliospores on water agar at 4°C in the dark, and second, incubating them at warmer temperatures for 1 week in the presence of an exogenous stimulator. Priming longer than 4 weeks resulted in significantly greater germination than priming for shorter periods. Sources of effective stimulator included seeds and seedlings of YST or safflower. The greatest germination occurred during incubation at 18°C in the dark. Teliospore germination was reduced after incubation with a 12- or 14-h photoperiod. A low percentage (<20%) of teliospores of two isolates germinated after 44 and 96 weeks of dry storage at room temperature; samples of each isolate tested after that did not germinate. Data indicate teliospores of several isolates of *P. jaceae* are viable, and the protocol will be used to prepare teliospores of *P. jaceae* for comparative studies with *P. carthami* on safflower seedlings.

Additional keywords: *Carthamus tinctorius*, *Centaurea solstitialis*, Uredinales.

Yellow starthistle (YST) (*Centaurea solstitialis* L.) is a weed pest of major importance in the western United States infesting more than 4 million ha in the state of California alone (16,18). Several insects have been released for biological control or YST, and additional agents are needed to attack foliage and bolting stems of the plant. One promising candidate is the rust fungus *Puccinia jaceae* Otth var. *solstitialis* (5,6).

During a risk assessment of Eurasian isolates of *P. jaceae* var. *solstitialis*, limited foliar infections occurred from urediniospore inoculations of safflower (*Carthamus tinctorius* L.). Infection of safflower by *P. jaceae* was minimal and limited primarily to cultivars highly susceptible to infection by *P. carthami* Cda., the cause of safflower rust (5). Modern safflower cultivars, which in general are less susceptible to infection by *P. carthami*, were rarely infected by *P. jaceae*. In every case, foliar infections by *P. jaceae* were insignificant compared with those caused by *P. carthami* under the same conditions (5). For these reasons, *P. jaceae* was judged not likely to pose risk to the safflower industry as a foliar pathogen if introduced for biological control of YST.

Safflower seedlings also are susceptible to *P. carthami*. Infections occur on the hypocotyls and may girdle or kill young safflower plants (20,21). This aspect of safflower rust comes after teliospore infestation of seed or soil. Concern was raised that *P. jaceae* teliospore infestations might also initiate a seedling disease on safflower. Viable teliospores of *P. jaceae* are needed to determine this; a protocol to germinate teliospores would enable the demonstration of spore viability, provide a measure of inoculum quality, and be useful in preparing spores for plant inoculations.

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There is no specific protocol for the germination of teliospores from any *Puccinia* spp. infecting plants in the genus *Centaurea*, and only a few protocols exist for the germination of teliospores of rust fungi in the Uredinales (1,9,11,13–15,17). Anikster (1) described a general protocol for germinating teliospores based on data from several species of *Puccinia*. Specific requirements have been described for germinating teliospores of *P. carthami* (13,14) and *P. punctiformis* (F. Strauss) Röhl., the cause of a rust disease on Canada thistle (*Cirsium arvense* [L.] Scop.) (11). Although safflower and Canada thistle are closely related to the *Centaurea* spp., the fungi that infect these hosts are distinct from *P. jaceae* (19).

This paper presents data on germination requirements of *P. jaceae* teliospores and describes a protocol for preparing teliospores for a risk assessment of *P. jaceae* var. *solstitialis* for biological control of YST.

MATERIALS AND METHODS

Teliospore sources. Teliospores of *P. jaceae* were from two general sources. Several field isolates, designated 95-308, 95-336, 95-339, 95-352, and 95-353, were collected in Iran by F. Eskandari in August 1995. The second source of teliospores occurred in the containment greenhouse after artificial inoculation of YST with urediniospores of *P. jaceae* isolate 78-06. This isolate was originally collected from Bulgaria by R. G. Emge in 1978 and maintained on YST by urediniospore inoculations. All teliospores in this study were stored at room temperature on dry YST leaves and stems. Identification of accessions as *P. jaceae* was based on urediniospore morphology (19).

General procedures. Teliospores were scraped from pustules and suspended in 0.1% water agar (12) containing 3 drops of Tween 20 (polyoxyethylene sorbitan monolaurate) wetting agent per 100-ml volume (WAWA). Teliospores were centrifuged for 1 min in a bench-top microfuge (BHG Hermle, National Labnet Co., Woodbridge, NJ) at the high setting to break surface tension

of the spores. Teliospores were surface-sterilized by adding an equal volume of 2% commercial bleach (for a final concentration of 1% bleach or 0.05% NaOCl). Spores were treated with bleach for 1 min followed by centrifugation for 1 min. They were rinsed with two cycles of sterile water and centrifugation to control growth of facultative organisms, particularly bacteria, on water agar plates. Spore concentration was adjusted to 30 to 60 teliospores per µl based on counts of teliospores in 1 µl of suspension.

All tests were conducted on water agar in snap-cap 50-mm petri dishes (Falcon 1006; Becton Dickinson and Co., Franklin Lake, NJ). Three 1-µl drops of teliospore suspension in WAWA were placed off center in a row on each plate of water agar. Stimulator sources were placed on the agar opposite the drops of spore suspension. Generally, tests of teliospore germination involved a two-step process that involved a cold treatment (the priming step) and a warm treatment with a source of stimulator (the incubation step). Teliospores on water agar were primed at 4°C in a refrigerator for predetermined periods. Then plates were removed from the cold after the priming period, supplied with a source of stimulator (seeds or 2-day-old seedlings), and incubated for 7 days at a warmer temperature, depending on experimental objectives. Unless otherwise noted, plates were primed for a minimum of 4 weeks and incubated at 15 or 18°C in the dark. Experiments testing effects of light were conducted in growth chambers under a combination of cool white fluorescent and incandescent lights with a 12- or 14-h photoperiod.

Data collection and statistical treatments. Germinated teliospores were counted in all 3 drops for each treatment combination. Teliospores were considered germinated when a well-developed basidium, at least as long as the teliospore, grew from either or both cells of the spore. Germination ratios were calculated for each drop. Data on germination ratios were transformed to arcsine equivalents for statistical analysis. Germination ratio of each drop was the statistical unit for analysis.

Independent treatments were subjected to analysis of variance using the general linear models (GLM) procedure of SAS (release 6.12, SAS Institute, Cary, NC). Least square means were estimated using the LSMEANS procedure in SAS, and probabilities for statistical similarity between means were calculated using the PDIFF option of LSMEANS within GLM. Means were considered statistically different if the probability that they were equal (PDIFF) was less than or equal to 5% ($P \leq 0.05$). Data for treatments representing continuous variables were analyzed by regression analysis within GLM. In studies on stimulator biomass and teliospore germination, analysis of covariance was performed with biomass as the continuous variable. Data points in which YST and safflower biomass were equivalent were compared on the basis of confidence intervals (CI) at $P = 0.05$.

Priming period. The effect of priming period was tested first with isolate 95-339 by placing teliospores on water agar in a refrigerator and removing a plate each week to incubate. In the first experiment, the stimulator source was a combination of one safflower seed and one half of a safflower leaf per plate. Plates were incubated for 7 days in the laboratory (fluorescent lights at 20 to 23°C). Comparisons were made with teliospores of *P. carthami* and by priming a set of plates of each *Puccinia* sp. at 30°C.

This experiment was repeated with two other isolates (95-353 and 78-06) and two safflower seeds per plate as the stimulator source. Priming period in the latter experiment was up to 10 weeks long.

Stimulator sources. The first successes in germinating *P. jaceae* teliospores involved stimulators from safflower. Three subsequent experiments were run to determine effectiveness of YST as a source of stimulator. In the first experiment, an average of 30 surface-sterilized, ungerminated YST seeds (=0.076 g per plate) were compared with no-stimulator controls. Two isolates, 78-06 and 95-353, were tested, and 9 drops of suspension (20.9 ± 2.7 [mean ± CI, $P = 0.05$] teliospores per drop) were counted for each of the four treatment combinations.

This experiment was repeated with isolate 95-339 as a direct comparison of safflower and YST for sources of stimulator. Treatments were conducted with no treatment (controls), one safflower seed, 0.5-cm^2 safflower leaf, four YST seeds, or 0.5-cm^2 YST leaf. In addition, one set of plates was incubated in the dark at 18°C and the other was incubated at 23.5°C with a 14-h photoperiod. Three drops of suspension (30.1 ± 3.4 teliospores per drop) were counted for each of the 20 treatment combinations.

Effect of YST and safflower seedling biomass on *P. jaceae* teliospore germination was compared with isolate 95-353 in a third version of this experiment. Plates were incubated either at 15°C in the dark or under the regimen of 15/24°C night/day temperatures

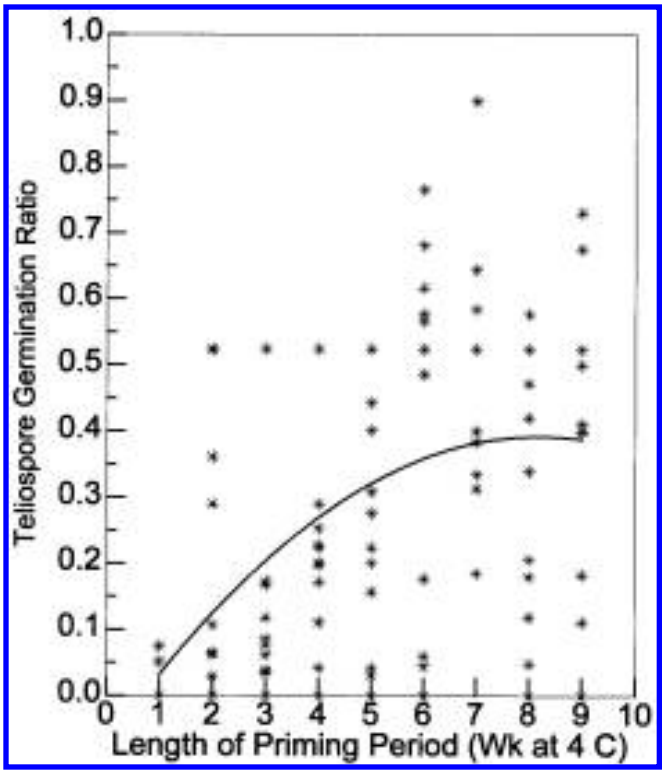

Fig. 1. Teliospore germination ratio (arcsine transformation of original data) as affected by length of time on water agar at 4°C (i.e., priming). Observed germination ratios for isolates 78-06 and 95-353 are plotted (stars). The regression curve for isolate data combined is $y = -0.085 + 0.105x - 0.007x^2$ ($r^2 = 0.175$), where y is teliospore germination ratio and x is time (weeks) of priming on water agar at 4°C.

TABLE 1. Least square means of *Puccinia jaceae* teliospore germination ratios (arcsine transformations of original data) after treatment with different stimulator sources and incubation at different temperature and photoperiod regimes[z]

Stimulator source	18°C/dark	22–24°C/light
Control	0.098 c	0.000 b
Safflower, 1 seed	0.788 a	0.083 ab
Safflower, leaf	0.143 bc	0.166 a
Yellow starthistle, 4 seeds	0.198 b	0.028 b
Yellow starthistle, leaf	0.000 c	0.015 b
Mean	0.245	0.058

[z] Teliospores were primed for at least 4 weeks at 4°C before stimulator source was added. Plates were incubated either in the dark at 18°C or in the laboratory with laboratory lighting (artificial photoperiod of fluorescent lighting for 8 to 10 h/day) at 22 to 24°C for 1 week after the addition of stimulator source. Numbers in each column followed by the same letter are not significantly different ($P \leq 0.05$).

and a 12-h photoperiod. Seedling biomass data were taken at the start and termination of the experiment and the change in biomass was calculated. Seedling biomasses (initial, final, and the change) were used as covariates in analysis of the results. Three drops of suspension (35.7 ± 8.7 teliospores per drop) were counted for each of the 16 treatment combinations.

Temperature. Two experiments were conducted on incubation temperature and teliospore germination. Isolate 95-339, with one safflower seed per plate, was incubated at 4, 10, 18, and 25°C in the dark, or at 25°C with a 12-h photoperiod. Teliospore germination was counted after 1 week. Plates from the 4 and 10°C dark and the 25°C photoperiod treatments were reincubated for an additional week at 18°C in the dark to verify teliospore viability. Three drops of suspension (32.9 ± 1.8 teliospores per drop) were counted for each of the five treatment combinations.

The first experiment was repeated over a more narrow temperature range with isolates 78-09 and 95-353 in attempt to clarify the optimum for *P. jaceae* teliospore germination. Plates were incubated with one safflower seedling for 1 week in the dark at 15, 18, 20.5, and 25°C. Two isolates, 78-06 and 95-353, were used in this study. Three drops of suspension (23.4 ± 1.3 teliospores per drop) were counted for each of the eight treatment combinations.

Photoperiod. Light effects on teliospore germination were evaluated in one experiment with three isolates from Iran (95-308, 95-336, and 95-352). Plates were incubated at 15/18°C night/daytime temperatures in a 12-h photoperiod or a 24-h dark (aluminum foil wrap) regime. Three safflower seeds were added to each plate as the source of stimulator. Three drops of suspension (26.4 ± 2.9 teliospores per drop) were counted for each of the six treatment combinations.

Two other experiments described previously also involved spores subjected to different regimes of light and temperature. Although light and temperature treatments were confounded in these particular studies, results were used to substantiate findings from the direct study on photoperiod (described previously).

Shelf life of teliospores. Germination of teliospores on water agar was recorded throughout the course of these and related studies involving inoculation of safflower seedlings. The highest rate of teliospore germination in each experiment was plotted against time (weeks) in storage. Analysis of covariance by isolate for length of storage was used to determine effects of time on teliospore viability.

RESULTS

Priming (vernalization). The first good germination of *P. jaceae* teliospores came after a 4-week priming period on water agar at 4°C followed by incubation in the presence of safflower seed and leaf material. Germination rate in plates primed for 1, 2, or 3 weeks ranged from 0 to 10.2% (±12.0) (mean ± CI, $P \leq 0.05$). Germination after 4 weeks of priming was 81.5% (±21.0). In comparison, germination of *P. carthami* teliospores primed over the 4-week period ranged between 54.9 and 92.2%. Control plates without stimulator had less than 1% teliospore germination, and teliospores of each species did not germinate when primed at 30°C for up to 4 weeks.

Repeating this with two other isolates for up to a 10-week priming period provided the same results. The isolate and the isolate–time interaction were not significantly different effects based on analysis of variance, and data for the isolates were combined for regression analysis. Average germination of teliospores was lower (<20%) when the priming period was less than 4 weeks. Germination improved over time and was greater than 35% after 6 weeks of priming based on the model (Fig. 1). Teliospores used in subsequent studies were primed for a minimum of 4 weeks.

Stimulator source. Germination of *P. jaceae* teliospores was stimulated in the presence of YST or safflower seedlings. Use of 31 ungerminated YST seeds per plate, which germinated during the course of the study, resulted in teliospore germination rates of 30.1 and 70.6%, respectively, for isolates 78-06 and 95-353. Each value was significantly greater than germination rates of 1 and 0% in the no-stimulator controls, respectively.

Importance of YST seeds for stimulation was substantiated in a similar study that compared seeds and leaves of either YST or safflower as sources of stimulator (Table 1). Environmental conditions also affected teliospore germination (Table 1). The highest rate of germination occurred with one safflower or four YST seeds and incubation at 18°C in the dark. Stimulation by a single safflower or four YST seeds in this regime was significantly greater at 18°C (78.8 and 19.8% germination, respectively) than either the no-stimulator controls (9.8%) or the equivalent treatments with leaf pieces (14.3 and 0%, respectively). Safflower leaves were equivalent to YST seeds as sources of stimulator in this experiment when incubated at 18°C in the dark. Germination at 23°C with a 12-h photoperiod was generally much lower (maximum of 16.6%) than equivalent treatments at 18°C in the dark; the highest rates were associated with safflower treatments.

Both the biomass of YST seedlings and the corresponding teliospore germination rates were much less than those for safflower in the previous study. For this reason, a study was designed to clarify effects of stimulator source at equivalent amounts of biomass

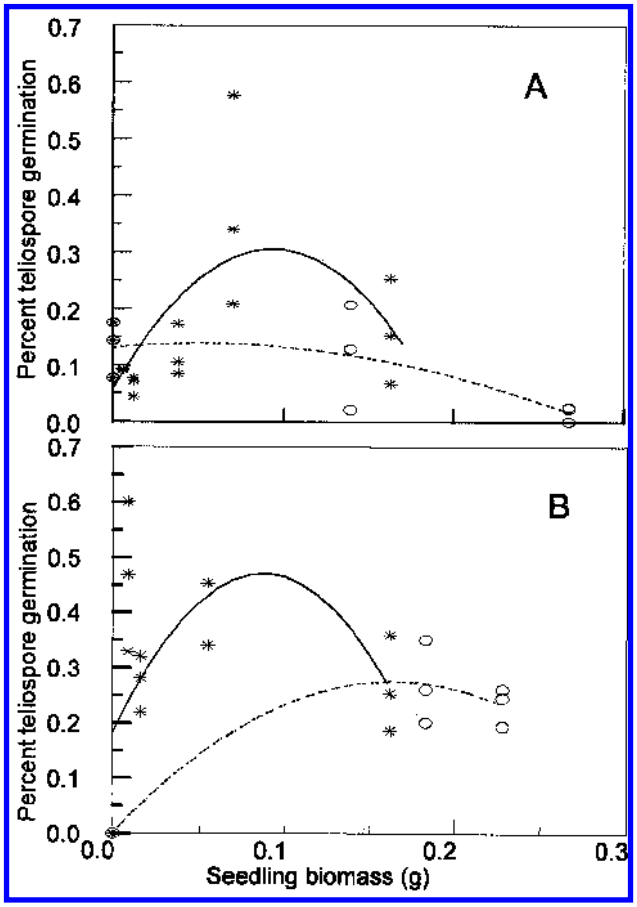

Fig. 2. Regression curves and observed values for teliospore germination ratios (arcsine transformations) as affected by stimulator from different amounts of initial seedling biomass (i.e., at the start of incubation) for yellow starthistle (YST) (stars and solid lines) and safflower (open circles and broken lines). Teliospores were incubated either at 15/24°C night/day temperatures and a 12-h photoperiod (graph **A**) or at 15°C in the dark (graph **B**). Regression equations for graph **A,** $y = 0.059 + 5.27x - 28.3x^2$ ($r^2 = 0.39$) for YST and $y = 0.132 + 0.27x - 2.6x^2$ ($r^2 = 0.51$) for safflower, and for graph **B,** $y = 0.18 + 6.64x - 37.9x^2$ ($r^2 = 0.24$) for YST and $y = 3.35x - 10.2x^2$ for safflower ($r^2 = 0.90$), where y is the germination ratio and x is the initial seedling biomass.

(Fig. 2). As before, incubation environment affected germination. Overall germination was lower when plates were incubated at 15/24°C night/day temperatures and a 12-h photoperiod (Fig. 2A) than when incubated at 15°C in the the dark (Fig. 2B). Germination ratios associated with YST treatments were generally higher than those from safflower treatments. The greatest germination, based on the regression curve in Figure 2B, was 47% under dark incubation at 15°C and an initial YST seedling biomass of 0.09 g compared with germination of 27.5% from safflower at 0.16 g. The greatest germination at 15/24°C and a 12-h photoperiod was 30.5% at an initial YST seedling biomass of 0.09 g, compared with germination of 14% from safflower at 0.045 g.

Mean germination and confidence intervals were calculated for data from each environmental regime where YST and safflower seedling biomass was closest. Germination rates for spores incubated at 15°C in the dark were 26.6% (±9.9) and 27.1% (±8.5) for YST at 0.162 g (initial biomass) and safflower at 0.183 g, respectively. Germination rates for spores incubated at 15/24°C with a 12-h photoperiod were, respectively, 15.7% (±10.5) and 11.9% (±10.6) from treatment with YST at 0.163 g and safflower at 0.139 g. These calculations suggest that under each incubation regime at more-or-less equivalent YST and safflower biomass, the stimulation of teliospore germination is not different.

Incubation temperature and light. Temperature and light during incubation affected the germination rate of *P. jaceae* teliospores. Germination of isolate 95-339 was 85.5% after incubation at 18°C in the dark, significantly greater than germination after incubation in the dark at 4, 10, or 25°C in the first experiment (Table 2). Normal basidia were produced at 4, 10, and 18°C, and basidiospores were most abundant at 18°C. Germination was 49.3% at 25°C in the dark, but only basidia were formed; basidiospores were not seen. Teliospores did not germinate when incubated at 25°C with a 12-h photoperiod. Reincubating plates from the 4 and 10°C/dark or the 25°C/light treatments at 18°C dark resulted in germination of 62.7, 80.0, and 0%, respectively.

In the second experiment with isolates 95-353 and 78-06, teliospore germination ratios (12.2 and 13.0%, respectively) were not different between isolates, and the isolate–temperature interaction was not significant. The best rate for isolates combined was 39.7% at 15°C incubation in the dark. Incubation at 18, 20.5, and 25°C in the dark resulted in lower average germination rates (Table 3).

Teliospore germination was inhibited by incubation with a 12-h photoperiod (Table 4). The treatment (light versus dark)–isolate interaction was significant in this experiment due to the extreme difference between means of isolate 95-308 compared with the others; for each isolate, regardless, germination under dark incubation conditions was always greater ($P \leq 0.05$). Average teliospore germination for the three isolates was significantly greater ($P = 0.0001$) when incubated at 15°C in the dark (28.1%) compared with germination of 4.8% at 15°C with a 12-h photoperiod (Table 4).

Teliospore germination was reduced under the combination of light and warmer temperatures. In two experiments described previously (Table 1; Fig. 2), conditions of light and temperature on teliospore germination were confounded. In each case, however, significantly greater germination occurred under cooler (15 or 18°C) dark incubation conditions than when teliospores were exposed, at least for part of the daily cycle, to light and warmer temperatures.

Teliospore longevity. A significant negative trend for teliospore germination of two isolates was observed for the length of time that teliospores were stored at room temperature (Fig. 3). Germination of the field-harvested Iranian isolate 95-353 was 20.6% (±4.4) after 96 weeks and 0.0% after 124 weeks. Viability of greenhouse-produced teliospores of isolate 78-06 declined much faster in comparison. Germination averaged 31.6% (±6.7) after 44 weeks and 0.0% after 63 weeks, about half the life span of isolate 95-353.

DISCUSSION

Germination of teliospores from several isolates of *P. jaceae* var. *solstitialis* was achieved in this study, thus verifying viability of the teliospores. Evidence from these studies suggests that there are two critical factors for stimulating teliospore germination of *P. jaceae*. These are priming under cold moist conditions followed by incubation at warmer temperatures with a source of stimulator. Other factors that may affect the rate of germination include length of the priming period, incubation temperature, light during incubation, length of storage, and concentration of stimulator.

These factors are not unique to the artificial germination of rust fungus teliospores. Anikster (1) found that presoaking (i.e., floating plant material with telia on water at 4°C for 2 weeks) enhanced germination of several species (not including *P. jaceae*). However, Anikster found that there was no germination of teliospores presoaked for 4 to 5 weeks. Presoaking is similar to priming in this study, except water agar was the substrate for teliospores in the present study. Our results for *P. jaceae* differed in that teliospore germination rate increased for up to 8 weeks of priming (Fig. 1), and in one instance, teliospore germination was 10.0% (±2.2) after a 25-week priming period with isolate 95-353. Flooding or submersion of teliospores greatly reduced the rate of *P. carthami* teliospore germination (13,15) and this also was observed for *P. jaceae* in the present study.

A source of external stimulator, either seedlings or seeds (that germinated during the experiment), was required to induce germination of *P. jaceae* teliospores in this study. In this aspect, germination requirements of *P. jaceae* teliospores were similar to those of *P. carthami* and *P. punctiformis* teliospores. Klisiewicz (13,14) showed that stimulators from direct exposure to safflower plant parts and their extracts, along with exposure to volatile chemicals derived from them, greatly increased germination of *P. carthami*.

TABLE 2. Least square means of *Puccinia jaceae* isolate 95-339 teliospore germination ratios (arcsine transformations of original data) and notes on condition of germinated spores after incubation at different temperatures[y]

Incubation regimen	Germination ratio[z]	Notes
4°C, dark	0.089 c	Normal germination
10°C, dark	0.045 c	Normal germination
18°C, dark	0.855 a	Many basidiospores
25°C, dark	0.493 b	Basidia/no basidiospores
25°C, 14-h photoperiod	0.000 c	…

[y] Teliospores were primed for at least 4 weeks at 4°C before stimulator source (one safflower seed per plate) was added.
[z] Numbers followed by the same letter are not significantly different ($P \leq 0.05$).

TABLE 3. Least square means of *Puccinia jaceae* teliospore germination ratios (arcsine transformations of original data) after incubation of two isolates at different temperatures and with a single safflower seed as a stimulator source[y]

	Isolate		
Incubation temperature	78-06	95-353	Average
15°C, dark	0.402 a	0.393 a	0.398 a
18°C, dark	0.013 bc	0.097 b	0.055 b
20.5°C, dark	0.105 b	0.000 b	0.052 b
25°C, dark	0.000 c	0.000 b	0.000 b
Isolate average[z]	0.130	0.122	…

[y] Teliospores were primed for at least 4 weeks at 4°C before stimulator source (a single 2-day-old safflower seedling per plate) was added. Numbers followed by the same letter in each column are not significantly different ($P \leq 0.05$).
[z] Averages for isolate are not statistically different ($P \leq 0.05$).

Stimulatory compounds from safflower were identified as C_{13} polyacetylenic hydrocarbons (3).

Similarly, French and his colleagues identified several sources of external stimulation for *P. punctiformis* teliospores, including a hexane extract from the roots of Canada thistle and several flavor compounds (7,8,10,11). The stimulatory compound from the hexane extract of Canada thistle roots was identified as a C_{13} unsaturated hydrocarbon (2).

Binder et al. (4) identified several volatile compounds from YST flowerheads, buds, leaves, and stems, including a variety of C_{13} polyacetylenic compounds. These were most concentrated in the buds and flowers, but occurred also in other above-ground plant parts. Roots were not extracted in their study. The compound or compounds responsible for stimulating teliospores of *P. jaceae* to germinate in this study have not been characterized.

Concentration of stimulator seems to be important, as suggested by data on biomass and germination (Fig. 2). Peaks in germination suggest there is an optimal concentration of stimulator; amounts less than the optimum are insufficient and greater amounts are, perhaps, relatively inhibitory. The data also suggest that use of YST seedlings under these conditions provided greater stimulator than safflower, but this is likely the result of concentration. A single safflower seedling may produce too much stimulator considering the similarity of germination rates at more-or-less equivalent biomasses of YST and safflower. The ultimate comparison between YST and safflower as sources of stimulator could be made if the stimulatory compounds from each plant species were characterized and tested at similar concentrations.

Optimal temperature for germination of *P. jaceae* in the present study was 15°C. This is similar to optima for germination of teliospores of most other rust fungi (1,17), including *P. punctiformis* and *P. chondrillina* Bubák & Syd. (7). Klisiewicz (13) reported that the optimum temperature for *P. carthami* teliospore germination on water agar was 24°C (ranging between 6 to 27°C and 15 to 24°C for two different isolates) with a 10-h photoperiod. The effect of total darkness on germination was not determined in the Klisiewicz study.

In the present study at 25°C under dark conditions, germination of *P. jaceae* teliospores was 43.9% but there were no basidiospores. Morin et al. (17) reported abnormal metabasidia and practically no basidiospores when *P. xanthii* Schwein was incubated at 28°C. In addition, in the present study, there was no germination at 25°C when the plates were given a 14-h photoperiod, and no teliospores germinated when the plate from that treatment was reincubated at 18°C in the dark.

In the Anikster study, light generally had no influence on rate of germination, except for *P. graminis* Pers. f. sp. *avenae* Eriks. Teliospore germination was suppressed during dark incubation of *P. graminis* f. sp. *avenae* in the Anikster study (1). In contrast, germination of *P. jaceae* teliospores was lower when incubated with a 12- or 14-h photoperiod compared with germination after incubation in the dark. Effects of temperature and photoperiod, although confounded, were consistent in two other experiments in which germination was significantly higher after incubation in the dark at cooler temperatures.

In the present study, viability of teliospores kept in dry storage at room temperature was retained for 1 year with one isolate and for 2 years with a second isolate. Anikster (1), in tests that included "most of the (twenty-seven) species" in his study (not otherwise specified), found that teliospores lost viability after 1 year of dry storage "outdoors" (temperatures not specified). Based on our experience and those reported by Anikster, teliospores seem to loose their viability within a year to two unless other measures are taken. Dry storage at 2 to 4°C prolonged viability for up to 3 years and dry storage at 5°C under partial vacuum in sealed glass vials extended the period of viability to 14 years (1). Storage of some isolates of *P. graminis* at –18°C preserved viability for at least 8 years (1). In support of Anikster's findings regarding refrigeration, teliospores of the safflower rust isolate used in this study had been stored dry and refrigerated for 4.5 years.

A protocol for preparing and germinating teliospores of *P. jaceae* has been developed based upon the findings in this study. Although there was considerable variability in teliospore germination within and between experiments, viability of teliospores in our collection has been demonstrated, and there is now a mechanism for testing viability of future accessions of *P. jaceae* teliospores. Successful germination of teliospores resulted from a minimum priming period of 4 weeks and incubation at 15°C in the dark for four additional isolates of *P. jaceae* var. *solstitialis* from Iran and for two isolates recently acquired from Uzbekistan.

Protocol from the results of this study will be used to prepare teliospores of *P. jaceae* for comparative studies with teliospores of *P. carthami*. Side-by-side studies with safflower seedlings infested with teliospores of each of these fungi will be used to determine if safflower seedlings become diseased in the presence of *P. jaceae*. Results from these inoculations are expected to complete the risk assessment of *P. jaceae* var. *solstitialis* for biological control of YST. The capability of germinating *P. jaceae* teliospores should enable development of a protocol for infection of YST foliage from teliospore inoculations as well.

TABLE 4. Least square means of *Puccinia jaceae* teliospore germination ratios (arcsine transformations of original data) after incubation at 15°C either in the dark or with a 12-h photoperiod (light)[x]

	Isolate[y]			
Treatment	95-308	95-336	95-352	Average
Light	0.068 a	0.052 a	0.024 a	0.048 a
Dark	0.502 b	0.172 b	0.168 b	0.281 b
Average isolate[z]	0.285 g	0.111 h	0.096 h	…

[x] Teliospores were primed for at least 4 weeks at 4°C before stimulator source (three safflower seeds per plate) was added.

[y] Numbers for each isolate and the treatment means that are followed by the same letter in each column are not significantly different ($P \leq 0.05$). The isolate–treatment interaction was significant ($P \leq 0.05$).

[z] Isolate mean values followed by the same letter are not significantly different ($P \leq 0.05$).

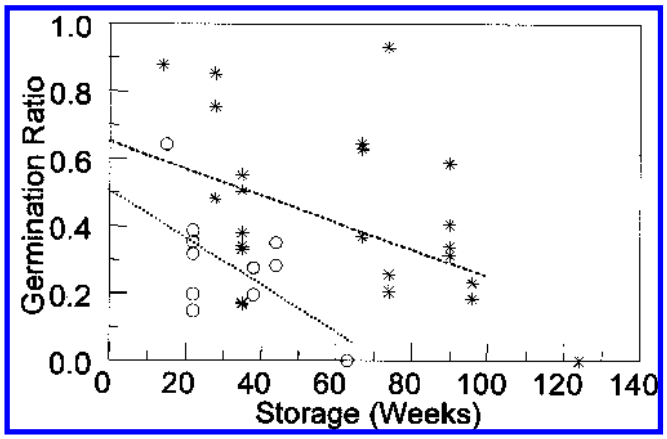

Fig. 3. Regression curves and predicted values for teliospore germination ratios (arcsine transformations) as affected by length of teliospore storage in telia on dry plant material at room temperature for greenhouse-produced teliospores (isolate 78-06; circles and broken line) and for field-collected teliospores (isolate 95-353; stars and solid line). Regression equation for isolate 78-06 is $y = 0.506 - 0.007x$ ($r^2 = 0.39$), and regression equation for isolate 95-353 is $y = 0.65 - 0.004x$ ($r^2 = 0.28$), where y is teliospore germination ratio and x is time (months) of dry storage at room temperature.

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LITERATURE CITED

1. Anikster, Y. 1986. Teliospore germination in some rust fungi. Phytopathology 76:1026-1030.
2. Binder, R. G., and French, R. C. 1994. Volatile components of Canada thistle. J. Agric. Food Chem. 42:2942-2945.
3. Binder, R. G., Klisiewicz, J. M., and Waiss, A. C., Jr. 1977. Simulation of *Puccinia carthami* teliospores by polyacetylenes from safflower. Phytopathology 67:472-474.
4. Binder, R. G., Turner, C. E., and Flath, R. A. 1990. Comparison of yellow starthistle volatiles from different plant parts. J. Agric. Food Chem. 38:764-767.
5. Bruckart, W. L. 1989. Host range determination of *Puccinia jaceae* from yellow starthistle. Phytopathology 79:150-160.
6. Bruckart, W. L. 1999. A simple quantitative procedure for inoculation of safflower with teliospores of the rust fungus, *Puccinia carthami*. Plant Dis. 83:181-185.
7. French, R. C. 1983. Germination responses of several species of rust spores to 5-methyl-2-hexanone, isomers of ionone, and other structurally related flavor compounds. J. Agric. Food Chem. 31:423-427.
8. French, R. C. 1990. Stimulation of germination of teliospores of *Puccinia punctiformis* by nonyl, decy, and dodecyl isothiocyanates and related volatile compounds. J. Agric. Food Chem. 38:1604-1607.
9. French, R. C. 1992. Volatile chemical germination stimulators of rust and other fungal spores. Mycologia 84:277-288.
10. French, R. C., and Lightfield, A. R. 1990. Induction of systemic aecial infection in Canada thistle (*Cirsium arvense*) by teliospores of *Puccinia punctiformis.* Phytopathology 80:872-877.
11. French, R. C., Turner, S. K., Sonnett, P. E., Pfeffer, P., and Piotrowski, E. 1988. Properties of an extract from Canada thistle roots that stimulates germination of dormant teliospores of Canada thistle rust (*Puccinia punctiformis*). J. Agric. Food Chem. 36:1043-1047.
12. Hornby, D., and Ullstrup, A. J. 1965. Physical problems of sampling soil suspensions in the dilution-plate technique. (Abstr.) Phytopathology 55:1062.
13. Klisiewicz, J. M. 1972. Effect of host plant materials and temperature on germination of teliospores of *Puccinia carthami*. Phytopathology 62:436-438.
14. Klisiewicz, J. M. 1973. Effect of volatile substances from safflower on germination of teliospores of *Puccinia carthami*. Phytopathology 63:795.
15. Klisiewicz, J. M. 1977. Effect of flooding and temperature on incidence and severity of safflower seedling rust and viability of *Puccinia carthami* teliospores. Phytopathology 67:787-790.
16. Maddox, D. M., and Mayfield, A. 1985. Yellow starthistle infestations are on the increase. Calif. Agric. 39:10-12.
17. Morin, L., Brown, J. F., and Auld, B. A. 1992. Effects of environmental factors on teliospore germination, basidiospore formation, and infection of *Xanthium occidentale* by *Puccinia xanthii.* Phytopathology 82:1443-1447.
18. Pitcairn, M., O'Connell, R. A., and Gendron, J. 1998. Yellow starthistle: Survey of statewide distribution. Page 85 in: Biological Control Program Annual Summary, 1997. D. M. Woods, ed. Calif. Dep. Food Agric. Div. Plant Ind., Sacramento, CA.
19. Savile, D. B. O. 1970. Some Eurasian *Puccinia* species attacking *Cardueae*. Can. J. Bot. 48:1533-1566.
20. Schuster, M. L., and Christiansen, D. W. 1952. A foot and root disease of safflower caused by *Puccinia carthami* Cda. Phytopathology 42:211-212.
21. Zimmer, D. E. 1963. Spore stages and life cycle of *Puccinia carthami*. Phytopathology 53:316-319.